Growth of aerial roots on and relationship to plant performance in highland maize landraces from Mexico

Noah Kayafas

Advised by Dr. Kristin Mercer
Department of Horticulture and Crop Science
The Ohio State University

April 2021

Abstract

Crop diversity is a fundamental aspect of food security, allowing crops to adapt and evolve for the most efficient use of energy. As crops change from wild plants to more domesticated species, the plant adapts to environmental conditions like temperature and soil conditions (Mercer and Perales 2018). Some plants form symbiotic relationships with bacteria to achieve the nutritional needs of the plant. Legumes and some grasses like Sierra Mixe maize from highland Mexico would be an example of this adaptation. The purpose of this study is to identify highland maize landraces that can produce aerial roots and mucilage. A field study was completed with 16 varieties of maize which were analyzed for aerial root production and measured growth parameters. The analyses showed that aerial root production is a trait in multiple accessions of maize from Mexico, and that there are relationships between aerial root production and the growth of the plant. The implications of these findings could lead to research into the degree to which various maize landraces benefit from associations with nitrogen fixing bacteria.

Introduction

Genetic diversity within crops is a fundamental aspect of food security, allowing crops to adapt and evolve for the most efficient energy use. As crops change from wild plants to more domesticated species, plants adapt to environmental conditions like temperature and soil conditions for better energy use (Mercer and Perales 2018). Some plants form symbiotic relationships with bacteria to better suit the nutritional needs of the plant. Legumes would be an example of this adaptation. Van Deynze et al. (2018) identified Sierra Mixe as a maize variety that fixed atmospheric nitrogen using aerial roots which produce a thick gel called mucilage

(Van Deynze et al. 2018). The implications of this are significant because nitrogen fixation is rarely observed in grasses and cereals (Peiffer et al. 2013). The purpose of this study is to determine the degree to which other highland maize landraces can produce aerial roots and mucilage.

The domestication of crops over time has created a variety of diverse crops with traits that benefit the needs of humans (Meyer and Prugaanan 2013). This co-evolutionary process creates domesticated varieties which have ecological, economical, and cultural importance. Plant's domestication can reduce the plant's ability to survive in nature making crops nearly entirely dependent on human interference for survival. The diversity that this domestication creates is what can be seen in maize landraces where crops have been grown under different limitations while serving communities as a source of food (Purugganan, 2019).

Mexico, the center of origin and domestication for maize, harbors large amounts of valuable genetic diversity. This diversity can be seen, in part, as variation in the plant phenotype, some of which are unique adaptations to environmental stresses. An example of the diversity is with drought stress. Hayano-Kanashiro et al. (2009) analyzed two drought tolerant landraces (Cajete criollo and Michoacán 21) and compared their gene expression to a drought intolerant landrace's (85-2) gene expression when grown under drought and drought recovery conditions. The drought tolerant landraces exhibited more drastic changes in gene expression to adapt to drought conditions (Hayano Kanashiro 2009). Mexican farmers contribute to this diversity by saving their seed from year to year and sharing it with each other (Nadal 2000). There are an estimated 1.38×10^{11} genetically different maize plants that evolve under domestication each season (Mercer and Perales 2018). Certain landraces of maize are eventually adapted to growing in the environmental conditions of a specific region (Corral et al. 2008). Landraces from a specific locale in Mexico perform under a set of conditions that is unique to the area and therefore may display unique phenotypic traits (Mercer et al. 2008).

Landraces grown in low-nutrient soils evolve means for using nutrients more efficiently (Ferro et al., 2007). Landraces grown on land with 0 kg/ha of nitrogen applied as inorganic fertilizer were capable of producing larger grain yields and were less responsive than hybrid maize when nitrogen was either supplied or removed (Ferro et al. 2007). Still other plants find ways to enter into symbioses with microorganisms to utilize nitrogen fixed from the atmosphere. One unique response to this diversity is the presence of aerial roots growing from nodes on the stem of the crop. Further investigation illuminated a symbiotic relationship between the plant and bacteria to fix nitrogen from the air captured by the mucilage. Recently, researchers were able to cultivate Sierra Mixe maize that produced aerial roots coated in a thick mucilage (i.e. gel) which was shown to harbor nitrogen-fixing bacteria (Van Deynze et al. 2018). They demonstrated that the aerial roots were able to support nitrogen being fixed by the bacteria in the mucilage, and it was efficiently transferred throughout plant tissues (Van Deynze et al. 2018). The study proved that the Sierra Mixe landrace was capable of nitrogen fixation at rates (29%-82%) above that of a non-fixing plant (Van Deynze et al. 2018). This nitrogen fixation had not been previously reported at these levels in maize. Aerial roots in maize were previously suggested to be used for lodging and water uptake, and now prove to serve a role in diazotrophic activity. Brace roots are often present in maize but these higher aerial roots never reach the soil surface and are higher up the stem than most brace roots. This ability to fix nitrogen is very limited in cereals, and a development in the relationship between mucilage, bacteria, and atmospheric nitrogen is a very significant finding (Bennet et al..

This trait or suite of traits may have evolved to increase productivity of maize grown in the same soil every season, leading to poor overall soil quality (Van Deynze et al. 2018). Mucilage has been observed in other landraces of maize, grown in both Ohio fields and Wisconsin greenhouses, however, we still do not understand how common aerial root production, mucilage production, nitrogen fixation, and fixed N uptake are in landraces of maize. We do know that the aerial roots do not tend to be found in commercial varieties, but that may not be uniform.

Nitrogen is essential as a nutrient for many plants (Peiffer et al. 2013). For maize, it is usually given to the plant through inorganic fertilizers (Goodman and Brown 2015). Urea, a 46%N fertilizer, is a popular fertilizer used for adding nitrogen to soil environments (Goodman and Brown 2015). The fertilizer is subject to several reactions that can lead to the loss of N availability to the crop (Goodman and Brown 2015). If the fertilizer is applied during warm weather and is not incorporated into the soil or is exposed to the soil surface, there can be as high as a 30% loss in the applied urea N (Goodman and Brown 2015). There is also the threat of runoff loss that is both inefficient for the agricultural system and the surrounding environment (Goodman and Brown 2015). Less urea fertilizer could be used if the corn were fixing N from the atmosphere. If maize production systems instead had a miaze variety that was able to fix N, there would be less of a dependence on inorganic N fertlizers. The identification of nitrogen fixation in the mucilage of aerial roots is relatively recent, and it is not yet known how common aerial root production, mucilage production, nitrogen fixation, and uptake of nitrogen are in commercial varieties of maize but it also not known how common it is in landraces. It is also not known what truly causes this adaptation or what conditions need to be present for nitrogen fixation through mucilage on aerial roots.

Thus, in order to discern the degree to which nitrogen fixation may be occurring in other maize varieties, we planted 16 accessions of maize landraces, 15 from Mexico and 1 from the United States, in the field in Ohio to address the following three objectives:

I. Determine whether aerial root and mucilage production are found in other highland landraces from Mexico.

II. Assess to what degree aerial root and mucilage production are variable in their expression among and within landraces.

III. Measure the growth of landrace accessions to determine if early growth relates to aerial root production and how aerial root production impacts later growth.

The impact of our findings could contribute to the knowledge of landrace varieties that can form this symbiosis.

Materials and Methods

Maize landraces in Mexico are phenotypically diverse. There are hundreds of races of maize grown globally, with 59 grown in Mexico (Wellhausen et al. 1952). These different races are typical of unique environmental conditions (Corral et al. 2008). The conditions that these different landraces are grown in differ in elevation, temperature, and overall climate. The range of environments and elevations has created the diversity of Mexican landraces (Wellhausen et al. 1952).

Plant Material

In the 2019 field experiment in Columbus, we grew 16 accessions of maize. The field experiment was conducted in 2019 at The Waterman Agricultural and Natural Resources Laboratory on a plot of land that the OSU Student Farm allowed us to use. All but three of the accessions were from highland areas (>1800 m) of Mexico; the accessions that were not from highland Mexico were an outcrossing US variety initiated from 1930s varieties (Buffalo Seed Company, KS), CHIS, and CHIS 910 (Table 1). The other accessions were from highland areas of Mexico between 2009 m and 2746 m (Table 1). All landraces were obtained from CIMMYT (International Maize and Wheat Improvement Center, Texcoco, Mexico).

The one US variety was sourced from Buffalo Seed Company and was called Re-Pioneer. This is a diverse maize landrace that was started in Columbus, Ohio by mixing 20 pre-hybrid open pollinated maize varieties from the early 1900s. The population was grown without any selection, and the seed was collected and bred for three generations. The seed used in the experiment was from the F3 generation.

Experimental Design

The plot area was surrounded by a cover crop mixture of winter-kill peas, hairy vetch, crimson clover, and winter rye. The area was a 10m x 50m strip that was previously used as a maize research plot the summer before. The land was hand-weeded and then loosely cultivated using a broad fork two days before planting. There was a light cover of decaying plant material from the previous experiment that acted as a mulch cover once all the weeds were removed. We also utilized a black woven fabric as a physical means for reducing the area that would require weeding. The fabric strips were laid out between plantings of maize and ran the length of the plot. The only power equipment that was used for weed control was a weed whacker around the plants and on the border of the plot. We avoided disturbing the soil beyond the broad forking.

The design of the experiment was a randomized complete block design with five blocks. For planting, we tried to mimic a traditional method of maize planting from Mexico, where there are multiple plants growing out of the same planting spot (or *mata*). Seeds were therefore planted into the same hole in groups of three, 60 cm apart within the row and with 100 cm between rows. In each of the five blocks, we planted all 16 accessions in two adjoining matas (i.e. two matas = 1 plot) for a total of 6 plants per accession, per block. To make walking between the plots and blocks easier, we installed walking paths that were 100 cm wide. Between blocks there was a space of 5 feet (See Figure 1 for layout).

Once the field had been prepared, we planted the seeds of each accession with a dibble stick (1" by 1" sharpened wood dowel). As noted above, we planted a single plot by dropping three seeds into each of two holes. The plots were labeled by block, plot number (1-16), and accession. The holes were covered up and the seeds were allowed to germinate. Frequent weeding was required at this stage to ensure the maize would have a competitive advantage over any weeds that had grown in the field.

Data collection

Plants were observed as they grew. Once the had reached vegetative stage 2, as defined by the Iowa State staging guide, we placed colored tags on the stem of each plant to differentiate plants within a *mata* (Figure 2) (Licht, 2016). These tags stayed on until they were too small for the size of the stem and began to constrict the base of the plant. With the tags removed, we painted leaves on individual plants to differentiate among plants within *matas*. Paint was sprayed on leaves lower on the stem which were either dead or had begun to dry out to identify the plants within a subplot and make sure that the measurements for each plant were consistent for the whole season.

On each plant, we measured early and final height to the leaf collar on the newest leaf, stem diameter, leaf width, and the number of nodes along the stem that were producing aerial roots. Early height was measured 27 days after the seeds had been planted. The number of nodes with roots measurements were taken twice a week for the entire experiment. Stem diameter, final height, and leaf width were all measured at the end of the experiment. The number of nodes with aerial roots was determined by observing root production over course of the season. Nodes are visibly swollen points along the stem at which growth occurs. Some nodes never produced aerial roots. Nodes that did produce roots would first have a single aerial root break through the outer layer of the stem (Figures 3 & 4). When a root had emerged and passed the outside layer of the stem, it was counted as a node with aerial roots. A running total was kept for each plant for the number of nodes with aerial roots and the day that each node with aerial roots was first recorded. If roots produced any mucilage, we denoted the presence of mucilage with a 1-3 rating which was based on the amount of mucilage that was observed (Figure 6). To receive a rating of 1, there had to be mucilage present but it may be slightly dried out and not feel slippery to the touch. A rating of 2 was awarded if the roots were only partially covered with mucilage which was visibly wet and slippery to the touch. A mucilage rating of 3 meant that the roots were completely covered in mucilage.

Plants were also observed for the presence of anthocyanin and trichomes. Anthocyanin presence was seen as any purple coloration in the leaf tissue of the plant. The anthocyanin presence was recorded throughout the season and was a measure using a 0 for no purple coloration and a 1 for the presence of purple or red color in the leaf tissue. Many of the plants exhibited anthocyanin in the leaves. Trichomes were measured with the same binomial method and were counted as a 1 if there were trichomes on the leaf tissue that was either attached to the stem or the leaf tissue separate from the stem. Trichomes were checked throughout the season and when a plant began to exhibit the trait, it received a 1. If the plant never produced trichomes or anthocyanin, the plant received a 0.

Another challenge with collection of root counts was deciding if roots were aerial roots or if they were brace roots. Steffens and Rasmussen define brace roots as “Nodal roots that form above soil level as part of normal postembryonic development” and aerial roots as “roots that form from internodes” (Rasmussen 2019). Brace roots are common in corn cropping systems. These roots emerge from nodes within the first foot of the stem and eventually reach down to the soil where they help keep the plant upright. Aerial roots and brace roots look similar, and it is even hypothesized that aerial roots are just brace roots that have not made it down to the soil yet (Rasmunssen 2019). Because of the difficulty in making this distinction, the first two nodes of roots that were observed were not counted as nodes that produced aerial roots and were distinguished when doing statistical analyses. These first two nodes with roots either reached down to the soil or were completely surrounded by soil. Instead, they were noted as brace roots and the first node of visible roots above the first two were considered the first aerial roots (Figure 5).

Statistical Analysis

The plants in block five of our field experiment were stunted relative to the others due to soil variation within the field that we did not know about until after planting. As a result, all subsequent analyses use data from blocks 1-4.

Number of nodes with roots over time by landrace: Using Excel and the averages of the number of nodes with roots for each date within each accession, we plotted the number of nodes with roots over the days after planting.

ANOVA analyses: The data that was analyzed with ANOVAs in R can be found in Table 2. The data was pulled from a master excel file using the package "readxl". The other packages that were used were "dplyer, ggplot,, lme4, tidyverse, agricolae, multcomp, multcompView, and tidyr". The model that was run analyzed each measured variable across accessions and block. The model was created as a linear mixed effect model with the measured variable as a dependent variable. Block was a random effect and landrace was the treatment effect. The interaction between block and landrace was treated as a random effect. The analyses for trichomes and anthocyanin were performed using binomial data. These variables were either a 0 or 1 and the averages were composed of up to 6 plants within a plot. There were also values in the stem diameter that were deemed too high to have been realistic measurements. These 6 values were removed and replaced with "NA"

Transformations were utilized when running the ANOVA after checking the plots for the assumptions of the ANOVA. A square root transformation brought higher root count values closer to the rest of the values. Final height was the final variable that required a transformation. There were some plants that were difficult to measure because they were extraordinarily tall. Measuring the taller plants with taped, overlapping meter sticks could have resulted in multiple plants being recorded as taler than they actually were. The final height was therefore transformed using a log transformation. LSD mean separations were performed to analyze how the means of the measured variables differed from one another. The results from the mean separations were plotted to better visualize the differences in means for each accession. The code to generate means was run using the "emmeans" package. The means were then plotted with standard errors to show how the accessions differed. The means were plotted using the package "ggplot".

Regression Figures:

Regression figures were generated to see if the predictor variable, which is often final root count, predicts the response variable. The regressions were generated using the raw data. The predictor variables were either root count or early height. The response variables were leaf width, stem diameter, final height, and root count. The model was made using lmer and treats block as the random effect (lmer(response variable ~ (1|Block) + predictor variable). The regressions were then plotted using the package "ggplot".

Results

Aerial Roots

The analysis for aerial root presence showed that an accession was able to reach the 100% mark, with TLAX 502 having all of its plants produced aerial roots. TLAX 89 had 95.7% of its plants producing aerial roots, followed by TLAX 402 (95.5%) and MEXI 109 (91.7%) (Figure 8C). The average number of nodes for each accession was plotted with days after planting (May 15th) on the x-axis (Figure 7). All accessions put on an average of at least 0.5 nodes with aerial roots by the end of the growing season. The lowest accession was the CML 576 plant. Excluding CML 576, the lowest final root count was an average of 1.7 nodes of aerial roots per plant, followed by the CHIS accession. The cluster of plants above the average all begin producing roots around 55 days after planting and continue to put on roots at a steady rate until they start to slow down production around 82 days after planting. The plants continue to put on roots with averages increasing until the end of the season. Of particular interest is the PUEB 686 landrace, which had an average of 4.4 roots per plant. This was the highest average and it is 0.5 roots above the next highest average which is held by AGUC 18 at 3.9 roots per plant (Figure 7). Means were generated and plotted for the final root count (nodes with aerial roots) for each accession. The means for the final root count were made using the square root transformation to meet the assumptions of the ANOVA and the mean separation. The means ranged from below 0.120 roots per plant (CML 576) to 3.68 roots per plant (MEXI 673) (Figure 8A). TLAX 502 was the second highest final root count mean, with 3.36 nodes with aerial roots present at the end of the season. There were still a lot of plants that had 0 nodes producing aerial roots. Every landrace had at least one plant in the field that did not produce any aerial roots besides TLAX 502. The mean separation for root count with plants that did not produce any aerial roots omitted shows how plants that successfully put on roots were putting on nodes with aerial roots. The data was not transformed other than the omission of “0”. The means are all between 2.44 (TLAX 531) and

5.08 (MEXI 673) nodes with aerial roots (Figure 8B). The analysis for the percentage of plants that produced 3 or more nodes with aerial roots showed that MEXI 673 produced aerial roots on 81.8% of the plants (Figure 9D). AGUC 18 produced aerial roots on 63.3% of the plants. The lowest percentage for the presence of at least 3 aerial roots was CML 576 which had 14.3% of the plants producing aerial roots (Figure 8D).

Morphological and Growth Traits

In my analysis of six different variables, I found significant differences between landrace accessions in three of the variables (alpha < 0.05): leaf width, early height, and final height (Table 2). The significance of these growth metrics shows that there was variation between accessions in how those plants expressed the traits of stem diameter, leaf width, and height for both the early and final measurement. The widest leaves came from the CHIS 910 accession which had a mean of 79.7 mm for a leaf width (Figure 8J). The lowest mean leaf width came from the TLAX 501 accession which was 49.4 mm. The leaf width of individual plants often far surpassed the mean value for the accession which can be seen with the red data points above 100mm in the Old Improved accession and the data above the AGUC 18 accession's mean (Figure 8J). For the analysis of early height, the CHIS accession was tallest at 9.97 cm and the CML 576 accession was shortest by far at 4.0 cm with the second shortest accession being CHIS 910 at 6.81 cm (Figure 8E). CML 576 also recorded the lowest mean for final root count (Figure 8A). The analysis of stem diameter revealed that stems were all wider than 13.1 mm, which was the mean for the landrace CHIS. The next lowest stem diameter was recorded for TLAX 501 at 13.8 mm. The widest stems were on the landrace MEXI 109, which has a mean of 24.5 mm (Figure 8I). At the end of the experiment, the tallest accession was the CHIS 910 landrace which was 288 cm tall (Figure 8F). The shortest accession was TLAX 531 which came in at 170 cm. In all accessions, more than half of the plants were observed to have trichomes present (Figure 8H). The MEXI 109 accession produced trichomes on all of the plants in the study, with a mean of 1. TLAX 501 was close to a mean of 1 with its value reaching 0.957 and the value for TLAX 89 reaching 0.955. Much like trichomes, many of the plants were producing anthocyanin (Figure 8G). The only accession to reach the 100% mark where all plants were observed to have produced anthocyanin was TLAX 502. All accessions were above the 50% mark. The highest mean for anthocyanin production was TLAX 502, followed by PUEB 686 with a mean anthocyanin presence of 0.958. The lowest mean value was reported for MEXI 673 which was 0.727 (Figure 8G).

Relationships with Roots

The relationship between early height and root was positive (Figure 9A). For each cm of early height increase, the number of nodes with aerial roots at the end of the season was increased by 0.08 nodes. The relationship between root count and leaf width (mm) had a positive slope (Figure 9B). The slope is 1.7714 and shows that, as one additional root is added to the stem of the plant, the leaf width is predicted to increase by 1.7714 mm. The relationship between final root count and stem diameter (mm) was positive (Figure 9C). As each additional node with aerial roots was produced, the stem diameter was predicted to increase by 0.832 mm. The relationship between root count and final height was positive slope (Figure 9D). As plants continued to put on aerial roots, the plants also got taller. For each additional node with aerial roots, the plants were 8.207 cm taller. The relationship between early height and final height was not significant (Figure 9E).

Mucialge

There were individual plants within accessions that produced mucilage at different point during the season. The accessions that produced mucilage were AGUC 18, MEXI 662 and 109, Old Improved, TLAX 502, 531 89, and 402. The accessions that achieved a mucilage rating of 3 at any point were AGUC 18, MEXI 662, Old Improved, and TLAX 531. The remaining accessions were rated at a 2 and did not reach a rating of 1 or 3 at any point during the season. The mucilage expression was variable and we noted that after heavy rain events, more mucilage was observed. The mucilage was quick to dry out so seeing mucilage coating roots was rather rare.

Discussion:

The ANOVA analysis for the final number of nodes with aerial roots had an F value of 0.096, which is close to being significant ($\alpha \leq 0.05$ (Table 2). The accessions varied in their final root counts. There were examples of plants with 8 nodes producing aerial roots and within that same accession or even within the same mata there could be an individual plant with only 3 nodes producing aerial roots (AGUC 18 for example). There was variation in how aerial roots were expressed between accessions and there was also variation in how that trait was expressed within an accession. This was evident when walking through the field study, and it was only confirmed by the analyses. When the accessions were analyzed for the presence of aerial roots (Figures 8 C & D) many of the plants were found to have produced at least 1 node with aerial roots. Of the 16 total accessions, 9 produced a mean number of roots higher than 2 (Figure 8A). This means that there were at least two nodes with aerial roots present in more than half of the accessions that were analyzed. There were 2 accessions that produced a mean final root counts above 3, MEXI 673 and AGUC 18 (Figure 8A). These two accessions were able to produce at least 3 nodes with aerial roots per plant, on average. Many of the accessions produced trichomes and anthocyanin (Figures 8 G & H). Plants differed in their growth traits as leaf width, early height, and final height varied significantly between accessions (Table 2). There were relationships between root count and stem diameter, final height, and leaf width that all showed positive sloped and significance for the relationship (Figures 9 B, C, & D). Early height showed no relationship for its ability to predict the final height of plants (Figure 9 E). However early height was a successful prediction of the final root count (Figure 9 A).

The objective of this study was to identify if there were landraces of highland maize from Mexico that were able to produce aerial roots in a way that was similar to the roots exhibited by Sierra Mixe maize in previous experiments (Van Deynze et al. 2018). The results from this study show that there were multiple plants that are capable of producing aerial roots and that they were able to exhibit this trait when grown in a field trial on Ohio soil. 15 of the 16 landraces produced above 1.7 nodes with aerial roots throughout the season, on average per plant (Figure 7). We can say that the trait of aerial root production is not just unique to Sierra Mixe maize, and that there are many other landraces from highland areas of Mexico which can also produce aerial roots. These landraces are worthy of further research to understand the trait more completely.

An objective that became more established as the growing season continued, and the plants began to differentiate in the way they looked, was the relationship between the final root count and growth measures like leaf width, stem diameter, early height, and final height. Plants like CHIS 910, an accession from Chiapas (Table 1), exhibited a final height of 295 cm (Fig. 9E). This same landrace had a mean final root count of 2.02 nodes with aerial roots per plant (Fig. 9A). MEXI 673, an accession from the Amacameca municipio of Mexico (Table 1), produced a mean final height of 281 cm (Fig. 9E) with a mean final root count of 3.68 (Fig. 9A). These two accessions were the tallest accessions. We wanted to determine to what degree the growth metrics we observed could be predicted by the number of aerial roots on the stem. The regressions explore the relationship between the growth metrics of leaf width, stem diameter, and final height as predicted by the final aerial root count. All regressions for leaf width, stem diameter, and final height show a positive slope for the relationship between increasing root counts predicting a taller plant with wider leaves and a thicker stem. These relationships between the predictor of final root count and the response variables shows that as root count increased in the field, plants were also increasing in leaf width, stem diameter, and final height. This relationship provides insight to the relationship between these maize accessions putting on aerial roots and their growth parameters. As roots are put on, the plant may benefit from their presence. This was outlined in previous studies where the roots and their mucilage were further analyzed to determine how the roots and their mucilage was advantageous for the plant (Van Deynze et al. 2018). Isotopic (N) ($^{15}N_2$) was transferred from the mucilage on aerial roots to the chlorophyll and root tissue of the plants (Van Deynze et al. 2018.) If the (N) fixed by the mucilage is taken up by the aerial roots and the plant uses this (N) to make up for inadequate N sources in the soil, the relationship between the growth metrics and root presence that we measured would be preliminary data which could support the hypothesis of aerial roots and mucilage serving as a means of fixation and uptake of (N) from the atmosphere.

The variation that was seen within an accession could be due to both genetic and environmental factors. Our method of planting in matas was a unique approach to most field trials that plant in consistently spaced rows, with one plant in each location where seed was started. In our field layout, the plants were grouped into clusters of three and all three plants grew from that same place in the field. This led to plants being shaded out by their fellow landrace members, which may have contributed to variation in height, stem diameter, leaf width, and the plants overall competitiveness. It was common to see one plant within a mata growing much taller and putting on more roots than the others. It is possible that plants established and began to outcompete their surrounding plants for resources. For example, within Block 1 Plot 11, Accession AGUC 18 plants 1,2 and 3 were planted in the same mata. These plants were competing for light, space, water, and nutrients. Plant 1 was able to grow to 268 cm with a stem diameter of 22.5 mm, a leaf width of 68.7 mm and a final root count of 8. Meanwhile, plants 2 and 3 were being shaded out by plant 1, which posted an early heigh of 11cm compared to 8cm and 9cm for plant 2 and 3. Plants 2 and 3 posted a final height of 152 cm and 208 cm, with stem diameters of 14.6 mm and 20.7 mm, and a leaf width of 36.1mm and 48.6mm. The maize that we studied is also notoriously diverse and cherished for this diversity (Rojas-Barrera et al, 2019). The plants may have been exhibiting this diversity and also experiencing the effects of being planted much closer than normally seen in field studies.

Early height was analyzed as a predictor to final height to determine if the presence of aerial roots was contributing to the differences in height that were being observed in the field. There was no relationship discovered between the early and final heights which could show that there were other factors that had an impact on the final height of the plants. If early height had been able to predict the final height, then we would be able to say that plants which had not produced aerial roots yet, had their final heights predicted in the early growth stages. Instead, we found that plants' final heights were not predictable from early heights, which could imply that the addition of aerial roots allowed plants to reach larger final heights.

It is difficult to connect the addition of aerial roots to the increased growth of the maize plant because of two reasons. The first reason is that we grew these landraces in a temperate environment which does not mimic the conditions that the plants experience in highland Mexico.

In Mexico, the plants experience high amount of precipitation (1032 mm or 40.6 inch per year) which comes in as a light mist that coats the aerial roots and provides water for the thick mucilage (Tlaxcala climate: Average Temperature, weather by month, Tlaxcala weather averages - Climate-Data.org, 2021). The conditions for mucilage production may not have been ideal in Ohio, as rains were frequent in the start of summer, followed by long periods of little rainfall. The lack of mucilage means that the plants could not provide an adequate media for the diazotrophs to live in. The plants may have benefited from more consistent precipitation events which allowed for the production of mucilage and the formation of mutualistic relationships with N-fixing bacteria. More consistent rainfall events may have shown a more significant effect on aerial roots leading to larger plants.

Acknowledgements

This project would not have started without the vision and guidance of my research mentor, Kristin Mercer. I would like to thank her for inviting me to work in the Plant Evolutionary Ecology Lab and allowing me to take the lead on research that we both thought would be of interest. Not only did she have the vision for the project but she instilled the confidence in me as a student researcher. The help I received from Layne Connolly and Jack McCoy allowed me to save time in many places and their advice made me a better researcher. They have their own projects and were always willing to provide a hand ang give their input. Katie McNamara set a great example for me as we were both doing our undergraduate research at the same time. Without her lead, I would have spent much more time finding the best way to do something instead of following her lead. I also extend my gratitude to Vivian Bernau who was able to source the seed for us from CYMMT (International Maize and Wheat Improvement Center) I would like to thank Anna Baltisberger and Chee Ngui for their support. I would like to also thank Dr. Alexander Lindsey for providing advice and materials for the setup of the field experiment. I would like to thank the Ohio State Student Farm for providing me to space and assistance for the field experiment. Dr. Lindsey's feedback on the thesis and his serving on the defense committee are much appreciated. I also extend my gratitude to my academic advisor Dr. James Metzger, for helping me create a flexible schedule which could accommodate this research and for serving on the defense committee. I would like to finally thank my friends and my family for giving me their guidance and support. Thank you to my committee for attending the defense and helping me graduate with research distinction. This project provided me with experiences that I have already begun to learn from and for that, I am grateful.

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Tables and Figures

Table 1: Maize accessions grown in Columbus, Oh in 2019

#	Landrace	Country	State	Municipio	Elevation (m)	Race (Wellhausen)
1	AGUC 18	Mexico	Aguascalientes	Aguascalientes	2009	Conico Norteno
2	CHIS 910	Mexico	Chiapas	--	~1550	--
3	CML 576	Mexico	Mexico/Puebla	Puebla	176	--
4	CHIS	Mexico	Chiapas	Comitan	~1550	Comitco
5	MEXI 109	Mexico	Mexico	Toluca	2717	Conico
6	MEXI 662	Mexico	Mexico	Amecameca	2505	Chalqueno
7	MEXI 673	Mexico	Mexico	Amecameca	2505	Chalqueno
8	Old Improved	U.S.	Re-Pioneer	--	--	--
9	PUEB 542	Mexico	Puebla	Guadalupe Victoria	2514	Palomero
10	PUEB 686	Mexico	Puebla	Guadalupe Victoria	2514	Conico
11	TLAX 402	Mexico	Tlaxcala	Cuapiaxtla	2465	--
12	TLAX 499	Mexico	Tlaxcala	Huamantla	2746	--
13	TLAX 501	Mexico	Tlaxcala	Huamantla	2514	--
14	TLAX 502	Mexico	Tlaxcala	Cuapiaxtla	2465	--
15	TLAX 531	Mexico	Tlaxcala	Cuapiaxtla	2565	--
16	TLAX 89	Mexico	Tlaxcala	Miguel Hidalgo	2310	Conico

Figure 1

The field layout for one block of the experiment growing maize in Columbus, OH in 2019 at Waterman Agricultural and Natural Resources Laboratory. Each "*" represents a plant, with three in each planting spot (or *mata*). Each colored pair of 3 "*" represents a plot of one of the 16 maize accessions. Each block was randomized separately.

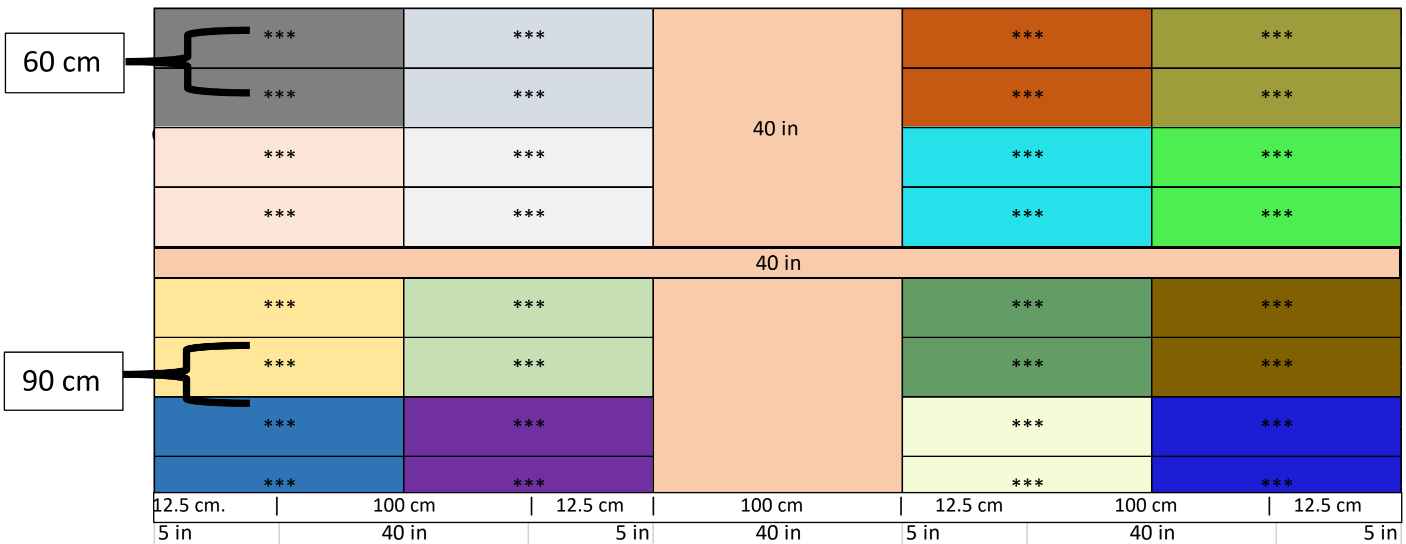


Figure 2
The plants were fixed with labels to separate the three plants within a *mata*.

Figure 3
A node which just broke the threshold to be considered a node that produced aerial roots.

Figure 4
A maize node that would not be considered to have produced aerial roots yet because they have not broken through the outer layer.

Figure 5

(a)
This node would be considered a node that produced brace roots.

(b)
This is a node with distinctive aerial roots about 50 cm up the stalk.

Figure 6
The different ratings for mucilage levels 1-3 are shown.

1/3 2/3 3/3

Table 2
Conglomerate table of ANOVA results

Alpha value = 0.05
All transformed variables are noted.
"." After a P value indicates that the value is near significant.
"*" After a P value indicates that the value is significant

Variable	**Sum of Squares**	**Mean Sq**	**Num DF**	**Den DF**	**F value**	**Significance**
Root Count (sq root transformation)	8.39	0.56	15	47.38	1.65	0.09694 .
Root Presence w/o "0"	31.19	2.08	15	37.19	1.44	0.1806
Root Presence (0/1)	2.05	0.14	15	45.57	1.74	0.076 .
% of plants w/ 3 Roots Present (0/1)	3.40	0.23	15	47.13	1.44	0.1697
Stem Diameter (mm)	289.43	19.30	15	48.45	1.24	0.2774
Leaf Width (mm)	4357.3	290.49	15	42.89	1.99	0.03965 *
Early Height (cm)	265.28	17.69	15	36.05	4.91	4.52 e-5 *
Final Height (cm) (Log transformation)	0.33	0.02	15	44.90	2.11	0.02759 *
Anthocyanin Presence	2.05	0.14	15	329.59	1.46	0.117
Trichome Presence	1.67	0.11	15	45.00	0.92	0.5462

Figure 7:
Number of Nodes with Aerial Roots Present After n Days Planting

Number of nodes with aerial roots over time on maize grown in Columbus, OH in 2019. Each line represents the average across all plants within an accession. Plants were no longer producing aerial roots after 120 days after planting.

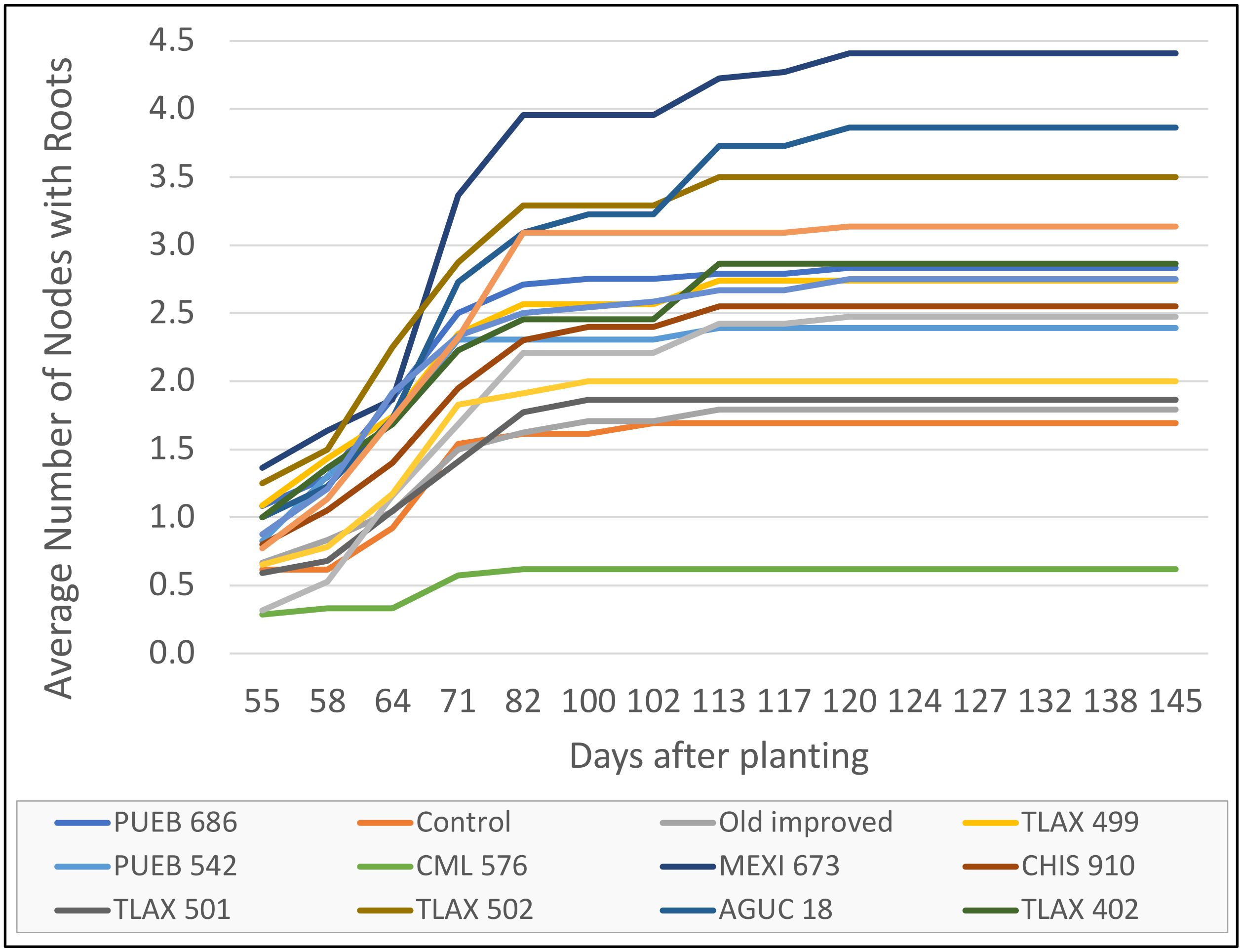
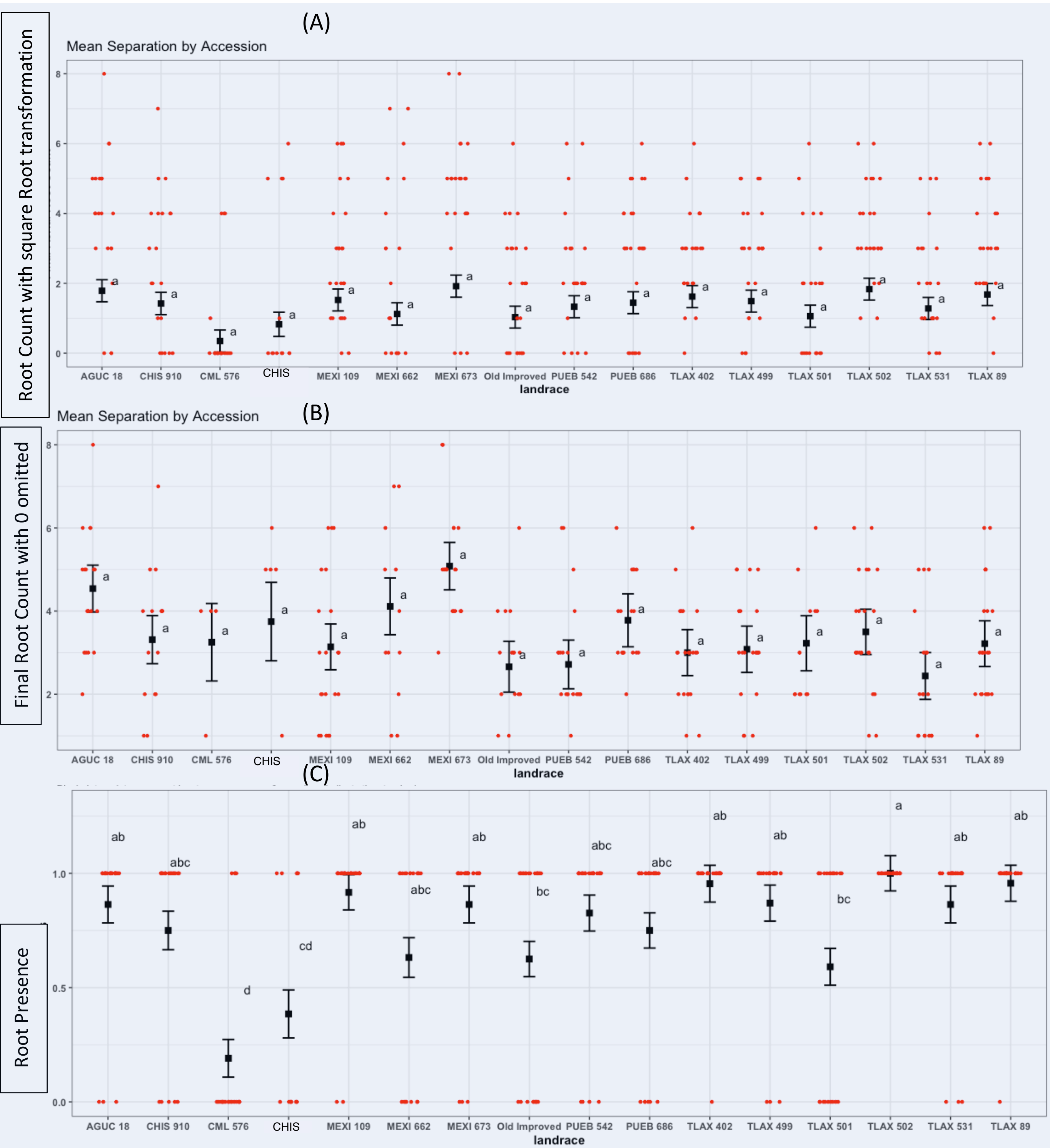
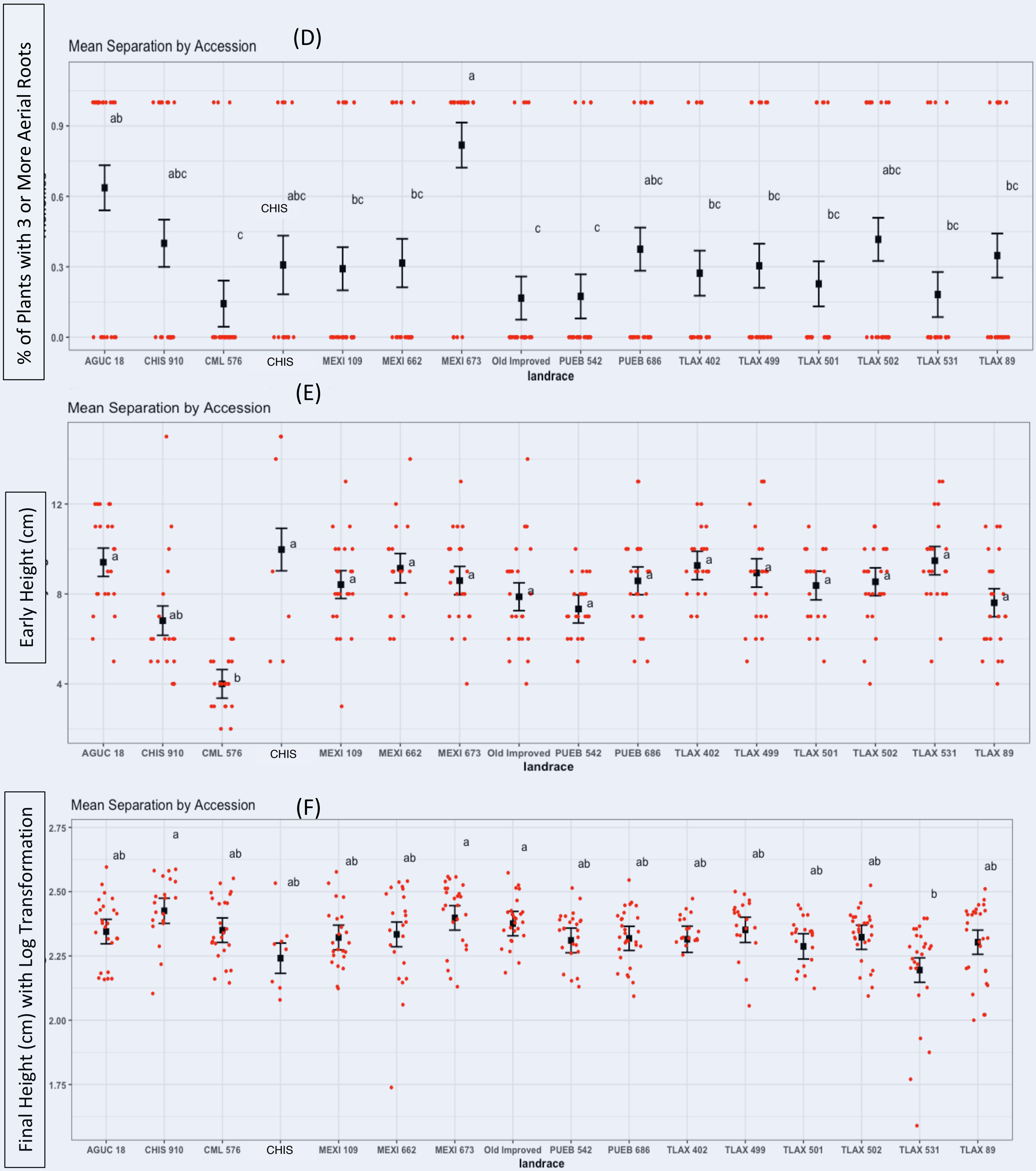


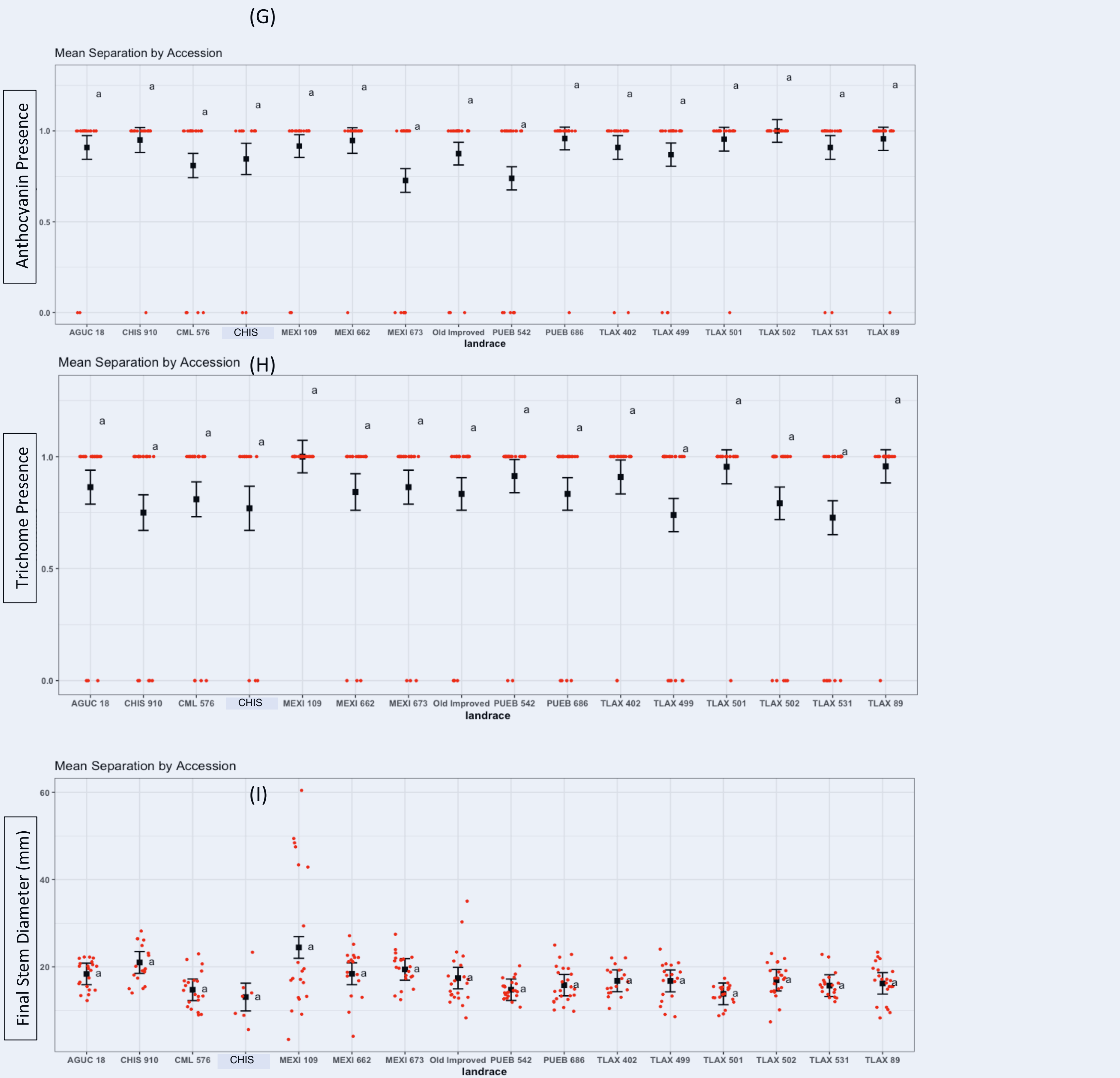
Figure 8
Mean Separations for all measured traits for all accessions

Traits measured on all accessions of maize grown in Columbus, OH in 2019. (A) Number of nodes with aerial roots; (B) number of nodes with aerial roots with zeros removed; (C) presence of aerial roots; (D) percent of plants with 3 or more aerial roots; (E) early height; (F) final height; (G) anthocyanin presence; (H) trichome presence; (I) final stem diameter; (J) final leaf width. Black data points represent least squares means & error bars indicate the standard error. Red data points show final aerial root count across all four blocks. Traits analyzed for presence, like trichomes, are percentages of the number of plants that exhibited that trait. Any transformations that were done are noted.


(A)
Mean Separation by Accession
Root Count with square Root transformation
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace
(B)
Mean Separation by Accession
Final Root Count with 0 omitted
landrace
(C)
Root Presence
ab
abc
d
cd
ab
abc
ab
bc
abc
abc
ab
ab
bc
a
ab
ab
landrace



(D)
Mean Separation by Accession
% of Plants with 3 or More Aerial Roots
0.0
0.3
0.6
0.9
a
ab
abc
abc
CHIS
c
bc
bc
c
c
abc
bc
bc
bc
abc
bc
bc
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace
(E)
Mean Separation by Accession
Early Height (cm)
4
8
12
a
ab
b
a
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace
(F)
Mean Separation by Accession
Final Height (cm) with Log Transformation
1.75
2.00
2.25
2.50
2.75
a
ab
b
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace



(G)
Mean Separation by Accession
Anthocyanin Presence
0.0
0.5
1.0
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace
Mean Separation by Accession
(H)
Trichome Presence
0.0
0.5
1.0
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace
Mean Separation by Accession
(I)
Final Stem Diameter (mm)
20
40
60
AGUC 18
CHIS 910
CML 576
CHIS
MEXI 109
MEXI 662
MEXI 673
Old Improved
PUEB 542
PUEB 686
TLAX 402
TLAX 499
TLAX 501
TLAX 502
TLAX 531
TLAX 89
landrace

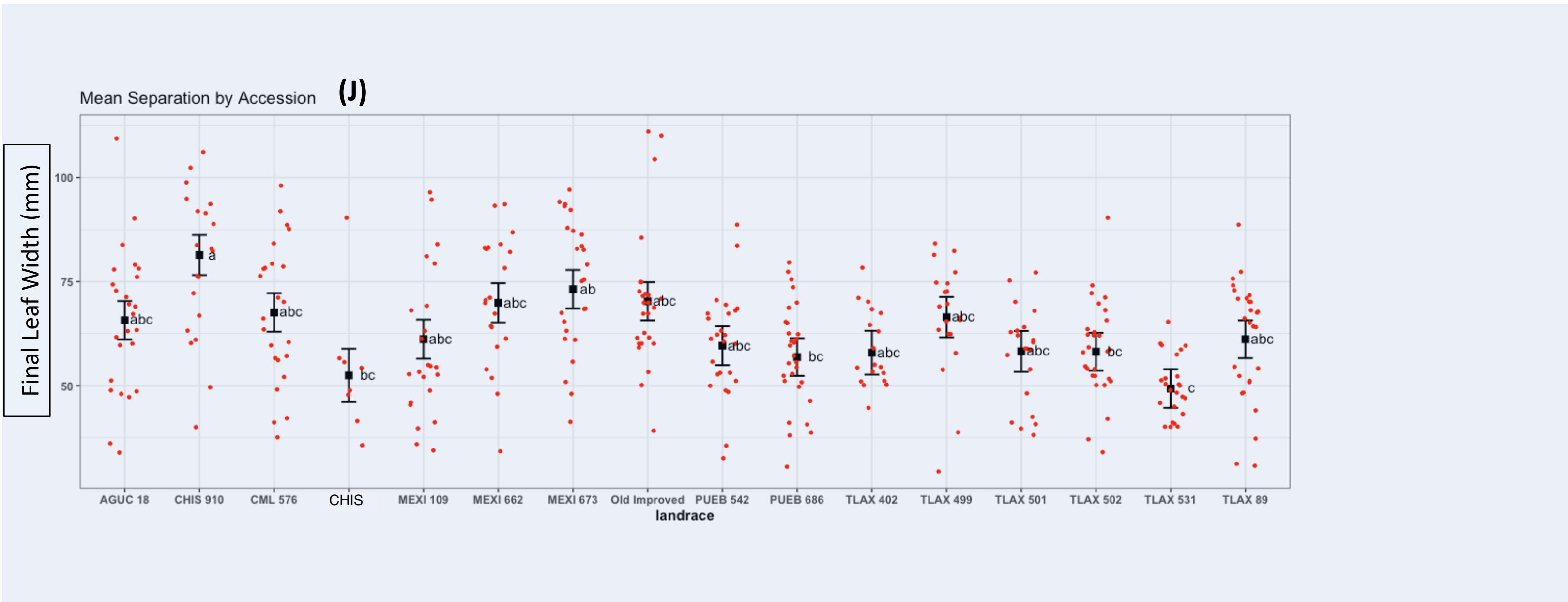
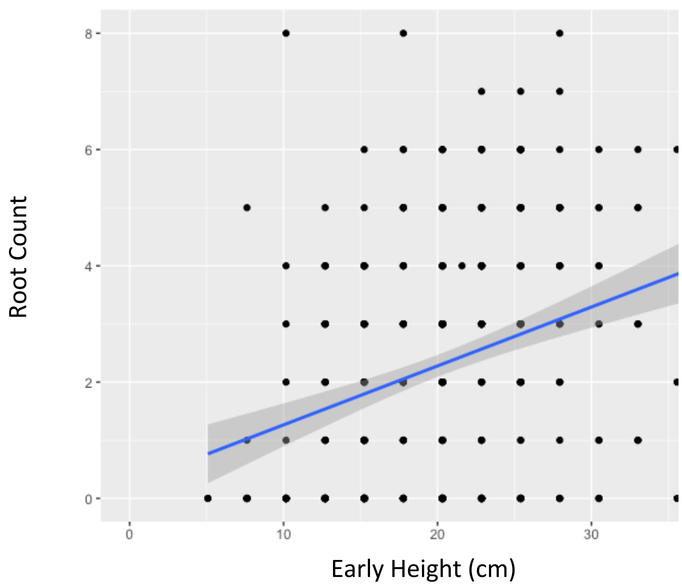


Figure 9
Regression Plots

Relationships between traits measured on maize grown in Columbus, OH in 2019. (A) Predicting number of nodes with aerial roots from early height (y = 0.07990x + 0.62825, p-value for slope: 2.1 x 10-7, R^2: 0.069). (B) Predicting leaf width with number of nodes with aerial roots (y = 1.7714x + 59.1384, p-value for slope: 3.84 x 10-5, R^2: 0.041612). (C) Predicting stem diameter with number of nodes with aerial roots (y = 0.832x + 18.3059, p-value for slope: 4.34 x10-8, R^2: 0.023). (D) Predicting final height with number of nodes with aerial roots (y = 8.170x + 217.3, p-value for slope: 9.43 X 10-7, R^2: 0.074). (E) Predicting final height with early height (y = -0.5612x + 233.7911, p-value for slope: 0.26, R^2: 0.002)

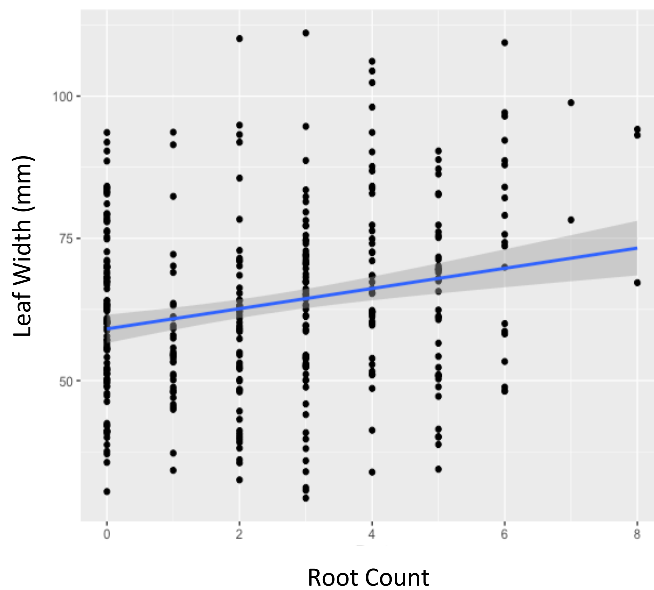
(A)



Legend:

Response Variable: Root Count
Predictor Variable: Early Height
Slope: y = 0.07990x + 0.62825
Significance: 2.1×10^{-7}
R-squared: 0.069

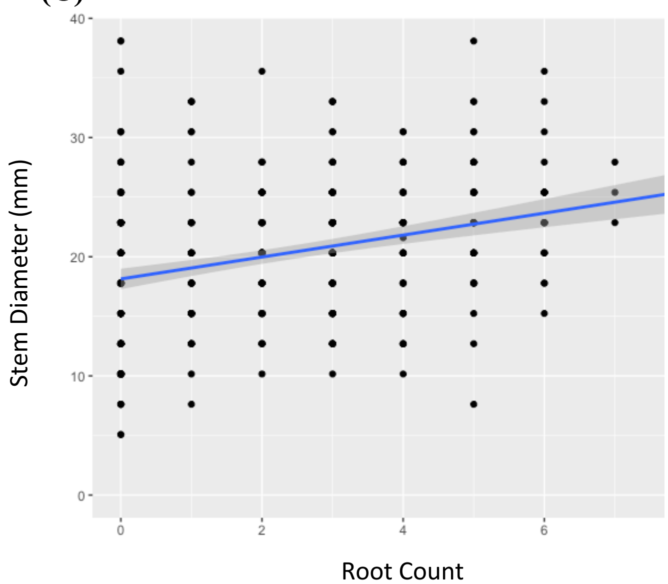
(B)



Legend:

Response Variable: Leaf Width
Predictor Variable: Root Count
Slope: y = 1.7714x + 59.1384
Significance: 3.84×10^{-5}
R-squared: 0.04612

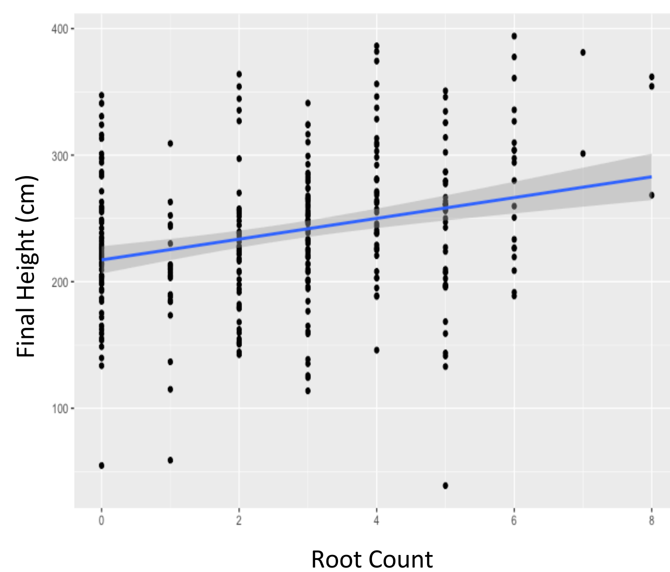
(C)



Legend:

Response Variable: Stem Diameter
Predictor Variable: Root Count
Slope: y = 0.832x + 18.3059
Significance: 4.34×10^{-8}
R squared: 0.023

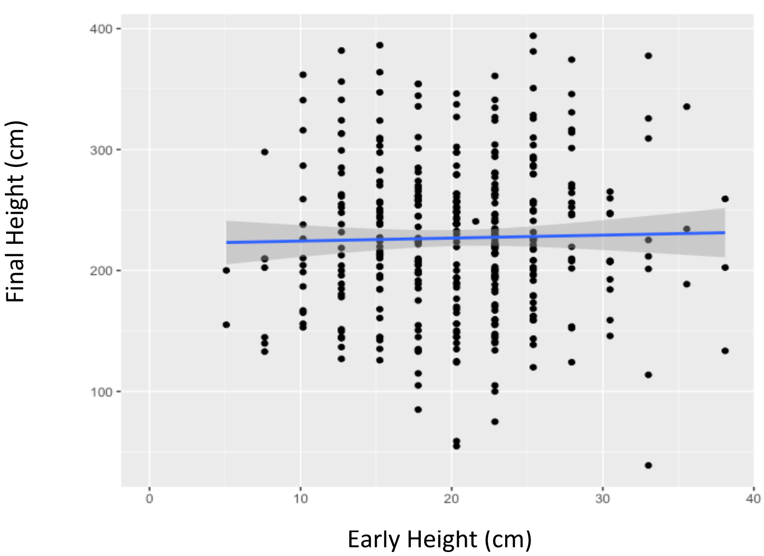
(D)



Legend:

Response Variable: Final Height
Predictor Variable: Root Count
Slope: y = 8.170x + 217.3
Significance: 9.43×10^{-7}
R squared: 0.074

(E)



Legend:

Response Variable: Final Height
Predictor Variable: Early Height
Slope: y = -0.5612x + 233.7911
Significance: 0.26
R Squared: 0.002